GROUP-SPECIFIC CARBOHYDRATE OF GROUP C-VARIANT HEMOLYTIC STREPTOCOCCI*, ‡

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The group-specific carbohydrates of hemolytic streptococci have been identified as major constituents of the cell walls. Attention has been directed to the chemical composition of the carbohydrates of Groups A and C streptococci and to the definition of determinants of antigenic specificity (1, 2).

In previous studies mutant strains of Group A, termed Group A-variant, were defined on the basis of a distinctive carbohydrate antigen (3). In this report evidence is presented which demonstrates that certain variant strains of Group C streptococci possess a carbohydrate antigen which is chemically and serologically similar to that of Group A-variant streptococci.

The Group A carbohydrate is composed of rhamnose and *N*-acetylglucosamine. Although Group C carbohydrate like that of Group A contains rhamnose as a prominent constituent the major hexosamine has been identified as *N*-acetylgalactosamine. Previous work supports the view that the antigenic specificity of Group A carbohydrate is dependent upon *N*-acetylglucosaminide residues terminal to rhamnose-rhamnose linkages (1), while the determinants of Group C carbohydrate specificity are *N*-acetylgalactosaminide residues terminal to the rhamnose moiety (2, 3). The Group A-variant streptococci are interesting in that the carbohydrate, composed almost entirely of rhamnose and nearly devoid of hexosamine, consists of rhamnose-rhamnose linkages which lack terminal amino sugar residues. Immunological evidence suggests that the rhamnose-rhamnose linkages are responsible for the immunological specificity of the A-variant carbohydrate (1). The carbohydrate of the Group C-variant streptococci like that of Group A-variant is also composed almost solely of rhamnose.

The present study suggests that the rhamnose moiety of the Group C-variant carbohydrate possesses rhamnose-rhamnose linkages immunologically similar

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to those of Group A-variant. These studies on the variant strains underscore the view that the carbohydrates of Groups A and C streptococci have a similar rhamnose structure but that the antigenic specificity is dependent upon the particular terminal *N*-acetylhexosamine.

Materials and Methods

The Group C-variant streptococcal strains 31C and 39C for the preparation of cell walls were obtained from Dr. Eugene Fox, Larabida Institute, University of Chicago. The parent Group C strain 26RP66 is from the author's strain collection.

The methods for preparation of streptococcal cell walls and the isolation of the group-specific carbohydrates have been previously described. The carbohydrate was extracted by the procedure of Fuller which employs hot formamide (5).

Analytical Methods.—Analyses for rhamnose, glucosamine, galactosamine, muramic acid, and amino acids were performed by previously described methods (6).

Quantitative Precipitin Tests.—Rabbit antisera reactive against group-specific carbohydrate were prepared as previously described (3). Quantitative precipitin analyses were performed by dissolving the washed antigen-antibody precipitates in 0.1 N NaOH and assaying the antibody spectrophotometrically at 287 mu (3).

EXPERIMENTAL

Group C-Variant Streptococci.—Group A-variant streptococci were recovered after serial passage in the mouse peritonium of the parent Group A strain (7). A similar procedure was employed in initial attempts to isolate Group C-variant streptococci. Group C streptococci were passed serially through the peritoneum of mice, and although strains were transferred more than fifty times, no variant strains were recovered from innumerable colony isolates. In another procedure devised to isolate variant strains, which also met with failure, Group C streptococci were passed serially in broth containing Group C antiserum. All isolations after many repeated passages were identified as Group C.

Variant strains of Group C streptococci, whose carbohydrate antigen had lost reactivity with Group C antiserum but had acquired reactivity with Group A-variant antiserum, were successfully isolated by a selection procedure which employed Group C bacteriophage. Although almost all Group C streptococci are lysed by Group C phage a very small number survive exposure. In some instances the survivors have been identified as variant strains as demonstrated by the fact that the carbohydrate antigen, although not reactive with Group C antiserum, reacts with Group A-variant antiserum. These strains have been termed Group C-variant streptococci.

In the experiments which follow evidence will be presented to show that these variant strains are clearly derived from the parent Group C strain and are not representative organisms of some other group of streptococci. The Group C-variant strains were supplied by Dr. Eugene Fox who isolated them in his laboratory.

Preparation of Specific Carbohydrate.—Hot formamide extraction of streptococcal cell walls results in two fractions; the soluble group-specific carbohydrate and the insoluble mucopeptide residue. An example of the extraction of Group C cell walls is given in Table I. The table includes the composition of the initial cell walls prior to extraction, the extracted group-specific carbohydrate, and the insoluble mucopeptide residue. The insoluble residue is com-

TABLE I

Composition of the Group C Cell Walls, the Soluble Carbohydrate, and the Insoluble Residue following Hot Formamide Extraction

	Cell walls	Formamide treatment		
		Extracted C CHO	Formamide residue	
	per cent	*per cent*	*per cent*	*mole ratio*
Rhamnose	20.3	43.0	1.0	—
Galactosamine	10.5	35.1	1.0	—
Glucosamine	5.8	3.9	8.2	1.2
Muramic acid	4.6	*	9.3	(1)
Alanine	16.6	*	25.1	7.6
Glutamic acid	7.5	*	12.1	2.2
Lysine	7.6	*	12.2	2.2
Glycine	2.1	*	2.8	1.0

C CHO, Group C carbohydrate.
* Less than 1 per cent.

TABLE II

Composition of the Group C-Variant Cell Walls, the Soluble Carbohydrate, and the Insoluble Residue following Hot Formamide Extraction

	Cell walls	Formamide treatment		
		Extracted C-variant CHO	Formamide residue	
	per cent	*per cent*	*per cent*	*mole ratio*
Rhamnose	31.5	86.0	4.3	—
Galactosamine	3.1	2.1	1.0	—
Glucosamine	6.2	4.2	10.4	1.7
Muramic acid	5.3	*	8.3	(1)
Alanine	16.25	*	22.6	7.6
Glutamic acid	7.1	*	10.1	2.0
Lysine	9.8	*	13.6	4.1
Glycine	0.34	*	0.66	0.3

C-variant CHO, Group C-variant carbohydrate.
* Less than 1 per cent.

posed of the constituents of the mucopeptide and the mole ratios of the elements are not unlike those of Group A streptococcal mucopeptide. The carbohydrate, free of the mucopeptide component, is composed primarily of rhamnose and galactosamine.

The results of a typical extraction of cell walls of Group C-variant strain 31C are presented in Table II. As in the case of Group C, two fractions are obtained by hot formamide treatment of cell walls, the soluble carbohydrate,

and the insoluble mucopeptide residue. The composition of the mucopeptide of Group C-variant resembles that of Group C streptococci. It is immediately obvious, however, upon a comparison of the data of Table II with that of Table I, that there are significant differences between the composition of the carbohydrates of Group C-variant and Group C streptococci. While the Group C carbohydrate contains 35.1 per cent galactosamine and 43.0 per cent rhamnose, the C-variant carbohydrate is composed of only 2.1 per cent galactosamine with the bulk of the substance consisting of rhamnose.

One feature of the chemical composition of the Group C-variant carbohydrate deserves special mention. As recorded in Table II a small percentage of galactosamine was detected in Group C-variant carbohydrate. Because galactosamine is not only a major but a characteristic component of Group C carbohydrate, the identity of a small percentage of galactosamine in the C-variant carbohydrate is indicative of the fact that the C-variant strains are derived from the parent Group C strains. Group A-variant carbohydrate, for instance, does not contain a similar percentage of galactosamine although a small quantity of glucosamine is present. The presence of both amino sugars in hydrolysates of the Group C-variant carbohydrate is illustrated by the paper chromatogram depicted in Fig. 1. Three components are evident, a rapidly moving one corresponding to rhamnose and two slower constituents which move with galactosamine and glucosamine. Chromatograms of hydrolysates of Group A-variant carbohydrate do not reveal a constituent corresponding to galactosamine.

The similarity of composition between Groups A-variant and C-variant carbohydrates is depicted in Table III. Approximately the same percentage of rhamnose is detected in the carbohydrate of both groups. In view of this similarity of composition between the two carbohydrates it is not surprising that C-variant carbohydrate cross-reacts with A-variant antiserum and gives only a minimal or trace reaction with Group C antiserum. These relationships are demonstrated by the quantitative precipitin tests depicted in Fig. 2. In the experiment illustrated on the left the C-variant carbohydrate gives a strong reaction with the A-variant serum while the Group C carbohydrate gives no appreciable reaction. The reverse situation is noted in the quantitative precipitin experiment illustrated on the right. The Group C-variant carbohydrate gives only a trace reaction with Group C antiserum, while the Group C carbohydrate gives the expected strong reaction.

A comparison of the quantitative precipitin reactions between A-variant and C-variant carbohydrates with three different Group A-variant antisera is depicted in Fig. 3. It is to be noted that in the precipitin tests performed with serum No. 917 both Group A-variant and Group C-variant carbohydrates gave identical precipitin reactions. In the quantitative precipitin reaction with serum 1115 the Group C-variant carbohydrate precipitates approximately

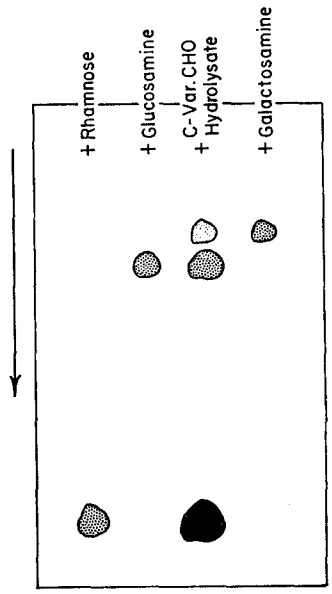

FIG. 1. Descending paper chromatogram of a hydrolysate of C-variant carbohydrate and standard sugars carried out for 48 hours with a solvent system of butanol-pyridine-water (6-4-2). The paper was developed with silver nitrate: acetone and sodium hydroxide: ethanol sprays.

TABLE III

A Comparison of the Composition between Group C-Variant and Group A-Variant Carbohydrates

	Carbohydrate			
	Group C-variant			Group A-variant
	Strain 39c Lot 1	Strain 31c		
		Lot 1	Lot 2	
	per cent	*per cent*	*per cent*	*per cent*
Rhamnose	86.5	87.5	86.0	85.0
Galactosamine	1.5	1.7	2.1	—
Glucosamine	3.0	3.4	4.2	3.0

one-half as much antibody as the Group A-variant carbohydrate. While this variation in the quantitative precipitin reaction suggests subtle differences between A-variant and C-variant antigens, in general it is clear that they possess similar determinants of antigenic specificity.

The reaction of the Group C-variant carbohydrate with A-variant antisera

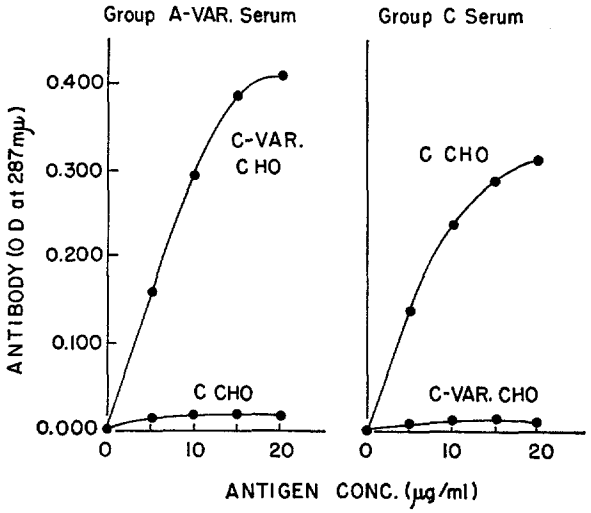

FIG. 2. A comparison of the quantitative precipitin analyses between Groups C (C CHO) and C-variant (C-var. CHO) carbohydrates with Groups A-variant and C antisera.

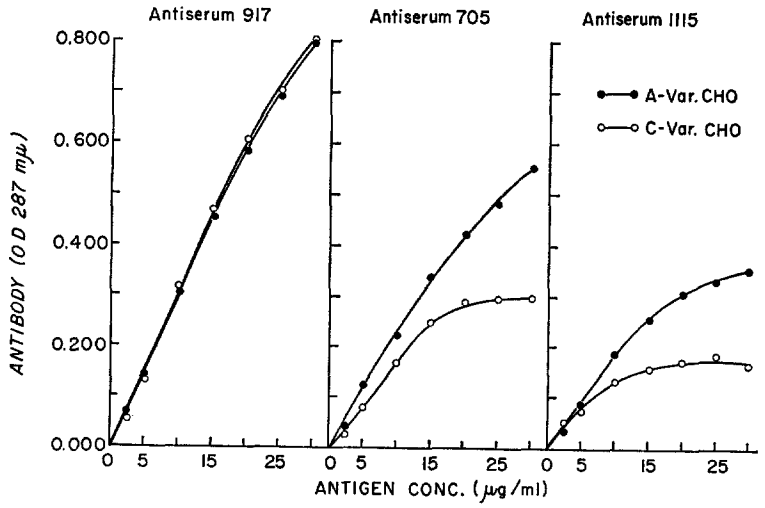

FIG. 3. A comparison of the quantitative precipitin reactions between Groups C-variant (C-var. CHO) and A-variant (A-Var. CHO) carbohydrates with A-variant antisera.

suggests that an antiserum prepared against Group C-variant streptococci would react with A-variant carbohydrate. Although C-variant antisera have not yet been prepared with sufficient potency to perform adequate quantitative precipitin tests, it is clear that they react with both C-variant and A-variant carbohydrates.

Previous studies of McCarty have defined the antigenic determinants of Group A-variant carbohydrate as rhamnose-rhamnose linkages (1). Evidence for this view rests in part on the fact that a rhamnosidase, an induced enzyme derived from soil organisms, destroys the antigenicity of the A-variant carbohydrate, releasing "rhamnose disaccharide" fragments which inhibit the precipitin reaction between the complete antigen and its antiserum. As would be expected the rhamnosidase also destroys the serological reactivity of the Group C-variant carbohydrate, thus supporting the view that Groups A-variant and C-variant carbohydrates possess similar rhamnose-rhamnose linkages as antigenic determinants.

TABLE IV

Composition of the Three Varieties of A Carbohydrate and C Carbohydrate, and the Serological Reactivity of these Antigens Prior to and after Treatment with Rhamnosidase

Carbohydrate	Composition			Reaction with antiserum			Reaction with antiserum after treatment with rhamnosidase		
	Rhamnose	Glucosamine	Galactosamine	A	A-variant	C	A	A-variant	C
	per cent	*per cent*	*per cent*						
Typical A	60.0	30.0		+	−	−	+	−	−
A-intermediate	67.9	15.2		+	+	−	+	−	−
A-variant	85.0	3.0		−	+	−	−	−	−
Typical C	43.0	3.9	35.1	−	−	+	−	−	+
C-intermediate	58.8	2.4	22.0	−	+	+	−	−	+
C-variant	86.0	4.2	2.1	−	+	−	−	−	−

DISCUSSION

These studies underscore the close antigenic relationship between Groups A and C hemolytic streptococci. This relationship is clearly demonstrated in the summary presented in Table IV which outlines the chemical composition and serological reactivity of Groups A, A-variant, C, and C-variant carbohydrates. In addition there is listed the carbohydrates of two strains termed A-intermediate and C-intermediate. These strains were designated intermediate because the carbohydrates reacted with Group A-variant antiserum and either Group A or Group C antiserum (3, 4). Immunochemical data indicate that the intermediate strains do not possess two types of carbohydrates but rather contain a single antigen reactive with both antisera. It should be noted that Group A-intermediate carbohydrate contains 15.2 per cent glucosamine, a value intermediate between the 30 per cent of Group A and the 3 per cent of Group A-variant. As would be expected the rhamnose content of Group A-intermediate

is higher than that of Group A and lower than that of Group A-variant. C-intermediate carbohydrate has 22 per cent galactosamine, less than the 35.1 per cent of Group C and greater than the 2 per cent of Group C-variant. Correspondingly, the rhamnose content of 58.8 per cent is intermediate between that of Group C and that of Group C-variant.

The work of McCarty indicates that terminal *N*-acetylglucosaminide residues on the rhamnose-rhamnose linkages are the determinants of Group A carbohydrate specificity (1). Group A-intermediate carbohydrate reacts with Group A-variant in addition to Group A antisera indicating that a portion of the rhamnose-rhamnose linkages are devoid of terminal *N*-acetylglucosaminide residues, a view supported by the fact that the glucosamine content of the Group A-intermediate carbohydrate is less than that of Group A. Exposure of the A-intermediate carbohydrate to the rhamnosidase releases a portion of the rhamnose and, as recorded in Table IV, there is concomitant loss of cross-reactivity with A-variant antiserum. Group A-variant carbohydrate, devoid of terminal *N*-acetylglucosaminide residues on the rhamnose-rhamnose linkages reacts only with A-variant antiserum and upon treatment with the rhamnosidase loses serological reactivity (1).

A similar course of events is noted with the Group C carbohydrates. Previous work suggests that terminal *N*-acetylgalactosaminide residues on rhamnose-rhamnose linkages are determinants of Group C specificity (2). Group C-intermediate carbohydrate reacts with Group A-variant serum in addition to Group C indicating that a portion of the rhamnose-rhamnose linkages are devoid of terminal *N*-acetylgalactosaminide residues. This is supported by the fact that the galactosamine content of the Group C-intermediate is less than that of Group C. Exposure of the C-intermediate carbohydrate to the rhamnosidase releases a portion of the rhamnose and destroys the cross-reactivity with the A-variant antiserum (4). Group C-variant carbohydrate devoid of terminal *N*-acetylgalactosaminide residues on the rhamnose-rhamnose linkages reacts only with the A-variant antiserum and upon exposure to rhamnosidase loses serological reactivity.

These chemical and immunological considerations of the carbohydrates of variant strains of Groups A and C streptococci suggest that the rhamnose moieties of the A and C carbohydrates possess similar rhamnose-rhamnose linkages. These linkages are exposed in the case of Groups A-variant and C-variant carbohydrates and are thus the determinants of variant specificity. In the case of Group A the rhamnose-rhamnose linkages possess terminal beta *N*-acetylglucosaminide residues thus confirming Group A specificity and at the same time masking the variant activity of the rhamnose moiety. The rhamnose-rhamnose linkages of the Group C carbohydrate possess terminal *N*-acetylgalactosaminide residues, a feature responsible for the Group C reactivity of the carbohydrate, but which, at the same time, masks the variant reactivity of the rhamnose moiety.

SUMMARY

The trypsinized cell walls of Group C hemolytic streptococci are composed of the specific carbohydrate antigen and a mucopeptide matrix. Certain phage resistant strains of Group C streptococci, isolated from organisms which survive exposure to Group C bacteriophage also possess carbohydrate and mucopeptide fractions, but the carbohydrate gives a precipitin reaction with Group A-variant antiserum and not with Group C antiserum. These strains have been termed Group C-variant. The C-variant carbohydrate contains 86 per cent rhamnose and only 2 per cent galactosamine, and is thus chemically and immunologically similar to Group A-variant carbohydrate. The evidence suggests that the antigenic determinants of Groups A-variant and C-variant carbohydrates are rhamnose-rhamnose linkages. These results strongly support the hypothesis that the carbohydrates of Groups A and C streptococci are composed of a similar rhamnose moiety but that the determinant amino sugar terminal to the rhamnose-rhamnose linkages in the case of Group A is *N*-acetylglucosamine whereas that of Group C is *N*-acetylgalactosamine.

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